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AUTHOR(S):

Kadokura, Satoru; Miyamoto, Takeaki; Inagaki,
Hiroshi

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Aqueous Gel Permeation Chromatography of Electrolytes and Polyelectrolytes. III. Determination of Selectivity in Counterion Binding to Polyelectrolytes

Satoru KADOKURA^{*1}, Takeaki MIYAMOTO^{*} and Hiroshi INAGAKI^{*}

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An aqueous GPC method was investigated to serve as a means of measuring the selectivity in counterion binding to polyelectrolytes. For this purpose, the competitive binding of alkali metal cations by heparin in mixed counterion systems was determined by using a Sephadex G-25 gel column. The binding data obtained were analysed in terms of a new parameter, which was defined as a measure of the counterion selectivity on the basis of Iwasa's condensation theory for polyelectrolyte-counterion interaction in the mixed counterion systems. It is shown that (i) aqueous GPC provides a simple but reliable method for measuring the counterion selectivity of polyelectrolytes, (ii) the counterion selectivity of heparin for alkali metal cations follows the order $K^+ > Na^+ > Li^+$, and (iii) the relative affinity of K^+ to heparin in a mixed counterion system of K^+ and Na^+ increases with increasing temperature.

KEY WORDS: Aqueous GPC polyelectrolyte/Counterion selectivity/Condensation theory/Heparin/Alkali metal cation.

INTRODUCTION

Aqueous gel permeation chromatography (GPC) has been widely used as a means of studying the interactions of small molecules with macromolecules in solution. Several experimental procedures have been proposed for this purpose¹⁻⁷⁾, though the same type of gel is used. In this case, the gel pore completely excludes the macromolecular species, but is freely accessible to the free small molecules. In a previous paper of this series⁸⁾ we reported that GPC was capable of an accurate examination on the colligative properties of polyelectrolytes, such as the Donnan salt exclusion parameter and osmotic coefficient. The GPC method is, in principle, identical to equilibrium dialysis using a double-compartment system, so that experimental data obtained by GPC are similar to those obtained by equilibrium dialysis. However, no GPC study has been performed so far to examine the selectivity for the binding phenomena of different counterions to polyelectrolytes.

The aim of this paper is to demonstrate a GPC procedure for determining the selectivity in counterion binding to polyelectrolytes. Using a Sephadex G-25 column, the relative binding of alkali metal cations to heparin was measured in the mixed counterion systems. The present experimental procedure is similar to that developed by Hummel and Dreyer¹⁾ for studying the interactions between small molecules and macromolecules. A new parameter,

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^{*}門倉 暁, 宮本武明, 稲垣博: Laboratory of Polymer Separation and Characterization, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611.

which is required to deduce the counterion selectivity from the competitive ion-binding data, was defined on the basis of Iwasa's theory for the solution of polyelectrolyte with different counterion.⁹⁾ The results will be discussed in terms of this parameter.

ANALYSIS OF BINDING DATA

Strauss et al.¹⁰⁻¹²⁾ have proposed a method for estimating the selectivity in competitive binding of counterions to a polyelectrolyte in solution. In this method, the relative degree of the binding of different counterions, say, X, Y, ... to a given polyelectrolyte is estimated in the following manner: A series of binding experiments relevant to each counterion were carried out in the presence of another counterion, such as tetramethyl ammonium ion, whose binding to the polyelectrolyte is very much lower than that of X and Y; then, the binding data obtained for X are compared with those for Y to deduce a parameter describing the relative degree of the binding abilities to these counterions. However, information obtained in this way is not directly concerned with the real competitive binding among these counterions. A direct method has been required to estimate the counterion selectivity.

Now we consider a conventional aqueous GPC procedure in which we have an aqueous solution containing a pair of counter-cations, M_1^+ and M_2^+ , a common anion (co-ion), and polyion, P; a gel column is equilibrated with an aqueous eluent containing M_1^+ , M_2^+ and co-ion only; then a small amount of polyion dissolved in the eluent is injected and eluted. Let the equivalent concentration of polyion be C_p , and let all the small mobile ions be monovalent, for simplicity. We denote the concentration of countercation and of co-ion as C_J ($J=M_1^+$ or M_2^+) and C_A^- , respectively, assuming that the polyion has monovalent and negatively charged groups. The equilibrium of the system is generally defined by the equality of the electrochemical potentials of the small mobile ions. Thus

$$(\gamma_j^o)(\gamma_A^o)C_J^oC_A^o = (\gamma_j^i)(\gamma_A^i)C_J^iC_A^i, \quad J=M_1^+ \text{ or } M_2^+ \quad (1)$$

where γ_j and γ_A are the activity coefficient of the cation and anion, respectively, and the superscripts o and i refer to the outside and the inside polyelectrolyte-free solution of the gel phase, respectively.

In GPC, the electrochemical potential of the small mobile ions in the inside solution of the gel phase should be equal to that in the eluent.⁹⁾ Thus, the righthand side of eq. (1) may be replaced by $(\gamma_j^e)(\gamma_A^e)C_J^eC_A^e$; i. e.,

$$(\gamma_j^o)(\gamma_A^o)C_J^oC_A^o = (\gamma_j^e)(\gamma_A^e)C_J^eC_A^e, \quad J=M_1^+ \text{ or } M_2^+ \quad (2)$$

where the superscript e refers to the eluent. Here we assume that

$$\gamma_{M_1^+}^e = \gamma_{M_2^+}^e \quad (3)$$

This assumption is valid for the cations of alkali metal series in a low concentration range, since the difference in activity coefficient between these cations is very small in pure aqueous solution. Combination of eqs. (1), (2) and (3) yields

$$\frac{C_{M_1^+}^e}{C_{M_2^+}^e} = \frac{(C_{M_1^+}^o)(\gamma_{M_1^+}^o)}{(C_{M_2^+}^o)(\gamma_{M_2^+}^o)} \quad (4)$$

For an aqueous polyelectrolyte solution, the counterion activity coefficient, which is

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markedly lowered due to the interaction between the counterion and polyion, differs for different counterion.¹⁵⁻¹⁸⁾ Hence in the outside solution of the gel phase, the activity coefficients of counterions are lowered to different degrees. Iwasa's theory for polyelectrolyte solution with different counterion¹⁰⁾ provides a required theoretical expression for the activity coefficient of the counterion in polyelectrolyte solution (outside solution of the gel phase), γ_J^0 ,

$$\frac{(\gamma_J^0)}{(\bar{\gamma}_J)} = \frac{(\gamma_{A^+}^0)}{(\bar{\gamma}_{A^+})} \times \frac{C_J^0 - (C_J^0)_c}{C_J^0}, J = M_1^+ \text{ or } M_2^+ \quad (5)$$

where $\bar{\gamma}$ denotes the salt correction term due to the small ion-small ion interaction¹⁵⁾ and $(C_J^0)_c$ the concentration of counterions condensed onto the polyion.¹⁸⁾ The salt correction term is given by the derivative of the excess free energy due to small ion-small ion interaction. In the present case, it is reasonable to assume that

$$\bar{\gamma}_{M_1^+} = \bar{\gamma}_{M_2^+} \quad (6)$$

Then, eq. (4) is rewritten as

$$\frac{C_{M_1^+}^e}{C_{M_2^+}^e} = \frac{(C_{M_1^+}^e) - (C_{M_2^+}^e)_c}{(C_{M_2^+}^e) - (C_{M_2^+}^e)_c} \quad (7)$$

According to the condensation theory for polyion-counterion interactions by Manning,¹⁶⁻¹⁸⁾ the value of $(C_J^0)_c$ is estimated in terms of a dimensionless charge density parameter, ξ , defined by

$$\xi = q^2 / \epsilon k T b \quad (8)$$

where q is the protonic charge, ϵ the dielectric constant of the solvent, T the absolute temperature, k the Boltzmann constant, and b the average distance between charged groups. When the charged groups of the polyion and the counterions are monovalent, the critical value of ξ is unity; when $\xi \leq 1$, no counterion condenses on the polyion; when $\xi > 1$, counterions condense to reduce the value of ξ to unity. Heparin, which consists of repeating tetrasaccharide units, contains seven anionic charges per repeating unit, so that $\xi > 1$.⁸⁾ In such a case, the fraction of condensed counterions per charged group, f , is equal to $1 - \xi^{-1}$.

¹⁸⁾ The condensed fraction is given by the sum of that of each counterion,^{12, 17, 19)}

$$f = (f_{M_1^+}) + (f_{M_2^+}) = 1 - \xi^{-1} \quad (9)$$

where $f_{M_1^+}$ and $f_{M_2^+}$ are the condensed fraction of M_1^+ and M_2^+ , respectively, defined by

$$f_J = \frac{(C_J^0)_c}{C_p^0}, J = M_1^+ \text{ or } M_2^+ \quad (10)$$

Combining eq. (7) with eqs. (9) and (10), the condensed fraction of each counterion is expressed in terms of ξ :

$$f_{M_1^+} = (1 + R)^{-1} \{ (C_{M_1^+}^0 / C_p^0) - R [(C_{M_2^+}^0 / C_p^0) - (1 - \xi^{-1})] \} \quad (11)$$

and

$$f_{M_2^+} = (1 - \xi^{-1}) - f_{M_1^+} \quad (12)$$

where

$$R = C_{M_1^+}^e / C_{M_2^+}^e \quad (13)$$

when the value of ξ is known, the condensed fraction of each counterion, f_i , can be calculated from eqs. (11) and (12), since the quantities, $C_{M_1^+}^e$, $C_{M_2^+}^e$, $C_{M_1^+}^o$, $C_{M_2^+}^o$ and C_p^o are estimated from GPC experiments. Thus a parameter is conveniently defined as a measure of counterion selectivity:

$$\delta = \frac{(f_{M_1^+})(\gamma_{M_2^+}^o)(C_{M_2^+}^o)}{(f_{M_2^+})(\gamma_{M_1^+}^o)(C_{M_1^+}^o)} \quad (14)$$

$$= \frac{(f_{M_1^+})(C_{M_2^+}^e)}{(f_{M_2^+})(C_{M_1^+}^e)} \quad (15)$$

The value of ξ is estimated from the measurements of the colligative properties of the polyelectrolyte solution such as the Donnan salt exclusion parameter, \bar{F} ; ¹⁶⁾

$$\bar{F} = \begin{cases} (1/2)(1 - 1/2\xi) & \xi \leq 1 \\ (4\xi)^{-1} & \xi > 1 \end{cases} \quad (16)$$

$$(17)$$

These values can be determined by GPC with a relatively simple technique, as reported previously.⁸⁾ For the sample heparin used in this work, the value of ξ has been estimated by GPC and known to be 2.2 at 25°C.⁸⁾ Then, eq. (9) gives 0.54 for the total fraction, f , of counter-cations condensed onto the heparin.

EXPERIMENTAL

Materials

All chemicals were of analytical grade and used without further purification. Deionized water was used throughout the experiments. A commercial sodium heparin derived from porcine intestinal mucosa, purchased from Nakarai Chemicals Co., Kyoto (Lot No. MIK 4583), having an anticoagulant activity of ca. 150 IU/mg, was used in this work. In order to remove low molecular weight impurities the heparin was dialyzed against deionized water. The dialyzed sample solution was passed through a sodium saturated CM-Sephadex C-50 cation exchange gel column and then freeze-dried. The average molecular weight of the sample heparin was estimated to be 1.1×10^4 by sedimentation equilibrium method in 1M NaCl at 20°C.⁸⁾ Sephadex G-25 gel was purchased from Pharmacia Fine Chemical Inc.

GPC Experiments

A series of GPC experiments were carried out on a Sephadex G-25 gel column (1.1 × 50 cm) provided with a thermostated water jacket. The column was equilibrated with an aqueous solution containing two kinds of alkali metal cations of known concentrations. The sample heparin was dissolved in the same solution as used for the equilibrium. A small volume of the sample solution was applied to the column and eluted. The elution was carried out by gravitational force at a flow rate of 60 ml/h. The effluent was collected on a weight basis to give 4.5g for each fraction. The concentrations of alkali metal cations in each fractions were determined with a Shimadzu Model AA-610S atomic absorption spectrometer. The concentration of heparin was determined by means of spectrometric titration with methylene blue.¹¹⁾

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms of heparin, Na^+ and K^+ , when sodium heparin was eluted with eluent containing Na^+ , K^+ and Cl^- on a Sephadex G-25 gel column at 25°C . Here, the salt concentrations in the eluent were 2×10^{-3} M for KCl and 2×10^{-3} M for NaCl, respectively. A pair of negative and positive peaks appeared with respect to K^+ concentration on the chromatogram while two positive peaks were observed with respect to Na^+ concentration. It should be noted that the first positive peak in the chromatogram of K^+ and Na^+ , which appears in the same retention range as that for heparin, does not directly reflect the amount of concentrations condensed or bound on heparin. The plateau between the first and the second peak in each chromatogram indicates that equilibrium was achieved in the competitive ion binding reactions in the column. The concentrations of K^+ , Na^+ and heparin in the outside solution of the gel phase at equilibrium, $C_{\text{K}^+}^0$, $C_{\text{Na}^+}^0$ and C_{P}^0 can be estimated from the concentrations in the fractions containing heparin.

When sodium heparin was eluted with eluents containing mixtures of KCl and LiCl, a pair of negative and positive peaks appeared for both K^+ and Li^+ concentrations on the chromatogram. However, no peak was observed for Na^+ concentration in the fractions involving heparin, indicating that the sodium heparin can be used as a sample polyanion for the mixed counterion system of KCl and LiCl. The salt form of the sample polyion has no direct concern with competitive ion binding data in a given mixed counterion system, when

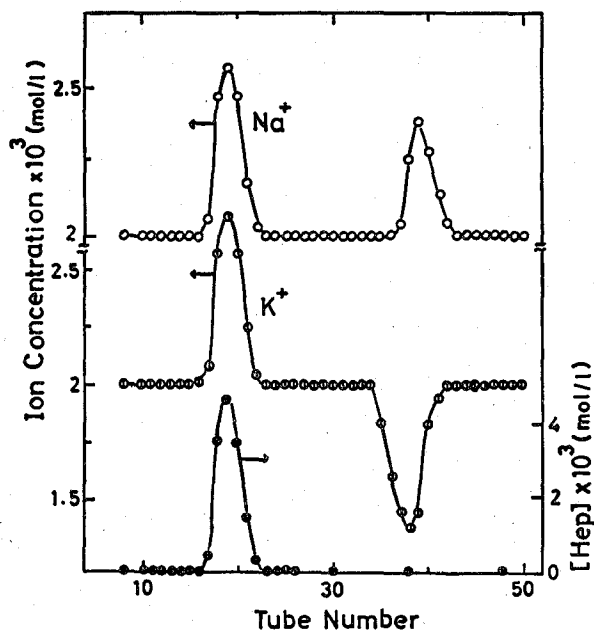


Fig. 1 Typical chromatograms of the system heparin-(KCl+NaCl) on a Sephadex G-25 column. Eluent: aqueous solution containing 2×10^{-3} M KCl and 2×10^{-3} M NaCl.

the experimental conditions are appropriate, as seen above. This may be one of the advantages of the present GPC method.

The condensed fractions of counter-cations, f_{K^+} and f_{Na^+} , were calculated using eqs (11) and (12). The calculation was made for each of the fractions containing the sample heparin. The values of f_{K^+} and f_{Na^+} were found almost independent of the heparin concentration in the fraction. Figure 2 shows the plots of f_{K^+} , f_{Na^+} and $\Delta f = (f_{K^+}) - (f_{Na^+})$ against the total salt concentration in the eluent, which is equivalent to the ionic strength of the eluent for the system heparin-(KCl+NaCl). The GPC experiments were carried out at 25°C under conditions, $C_{K^+}^0 = C_{Na^+}^0$. The results reveal that the ionic strength of the eluent has little influence on the condensed fraction of each counter-cation; the value of $\{(C_{K^+}^0) - (C_{Na^+}^0)\} / C_P^0$ is almost constant irrespective of the ionic strength of the eluent (see eq. 10).

Figure 3 shows the plots of f_{K^+} and f_{Na^+} against the composition of KCl in the eluent for the system heparin-(KCl+NaCl). The total salt concentration in the eluent was 2×10^{-3} M and the GPC experiments were carried out at 25°C. The selectivity parameter, δ , calculated using eq. (15) is also plotted against the composition of KCl in the eluent. The δ values were almost independent of the fraction of KCl. The average value of δ was determined to be 1.8 for the system at 25°C.

The GPC experiments were carried out for the heparin-(KCl+NaCl) system also at 15°C and 35°C. The average δ values estimated for each temperature are listed in Table 1. Table 1 also shows the average δ values for the systems heparin-(KCl+LiCl) and heparin-(NaCl+LiCl) at 25°C. These values were also determined under conditions, $C_{M_1^+}^0 = C_{M_2^+}^0$. From the δ values it can be seen that (i) the counterion selectivity of heparin follows the order $K^+ > Na^+ > Li^+$ for the alkali metal cations and (ii) the δ value for the system of heparin-(KCl+NaCl) increases with increasing temperature.

The selectivity order of the alkali metal counterion obtained here indicates the relative

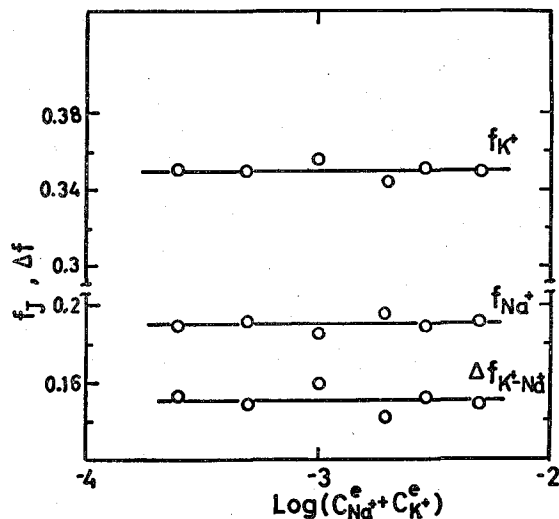


Fig. 2 Plots of f_{K^+} , f_{Na^+} and $\Delta f = (f_{K^+}) - (f_{Na^+})$ against the total ionic strength of the eluent in the system heparin-(KCl+NaCl) at 25°C.

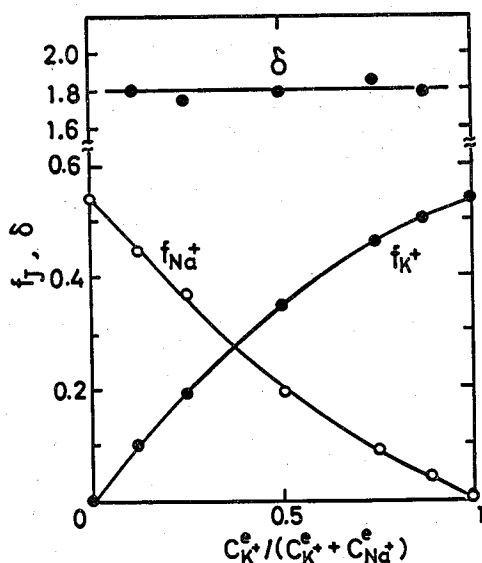


Fig. 3 Plots of f_{K^+} , f_{Na^+} and the selectivity parameter δ against the composition of KCl in the eluent containing the total salt concentration of 2×10^{-3} M for the system heparin-(KCl+NaCl) at 25°C.

Table 1 Condensed fractions of counterions on heparin and selectivity parameters in mixed counterion systems

System M_1-M_2	T°C	$f_{M_1^+}$	$f_{M_2^+}$	$\Delta f^{(a)}$	δ
K-Na	25	0.35	0.19	0.16	1.8
Na-Li	25	0.31	0.23	0.08	1.3
K-Li	25	0.39	0.15	0.24	2.6
K-Na	15	0.33	0.22	0.11	1.5
K-Na	35	0.36	0.18	0.18	2.0

a) $\Delta f = (f_{M_1^+}) - (f_{M_2^+})$

affinity of the ions to the polyanion, which is in good agreement with that obtained from the studies on the effect of alkali metal cations on the interaction between heparin and cationic dye.²⁰⁾ In connection with the result on heparin having $-\text{SO}_3^-$, $-\text{NHSO}_3^-$ and $-\text{COO}^-$ groups, it should be mentioned that the selectivity order of counterion of heparin for alkali metal cations is the same as that of polyvinylsulfonate¹²⁾ and dextran sulfate²¹⁾ but opposite to that of polycarboxylate.²²⁾

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